PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/004001	13.04.2004	15.04.2003

International Patent Classification (IPC) or both national classification and IPC
C12R1/465, C12P17/18, C12N15/76, C07K14/36

Applicant
GLAXO GROUP LIMITED

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☒ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/004001

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☒ The following document has not been furnished:

   ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☐ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 4,5,11(complete),and 6-9,12(partially)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 4,5,11(complete),and 6-9,12(partially)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-3,10 (complete),and 6-9,12(partially)

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-3,10 (complete) and 6-9,12(partially)
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-3,10 (complete) and 6-9,12(partially)
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-3,10 (complete) and 6-9,12(partially)
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rules 43*bis*.1 and 70.10)

   and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

   see form 210

Present application discloses a new process for the manufacture of clavams e.g clavulanic acid characterised in that the level of the expression of clavulanic acid is increased and the levels of the toxic 5S clavam is drastically decreased. Said method is based on the disruption and/or deletion of the reading frames of cvm6para, and/or cvm7para. Method for the production of clavam, polynucleotides as well as vectors and streptomyces clavuligerus microorganisms comprising said sequences are claimed.

Re Item IV

Lack of unity of invention

This Authority considers that there are 2 inventions covered by the claims indicated as follows:

a) Invention 1: Claims 1-3,10 (complete),and 6-9,12(partially)
Subject relating to the cvm gene cluster, respectively to the polynucleotide sequences as set forth in SEQ ID NO:1, SEQ ID NO:2 and SEQ ID NO:17.

b) Invention 2: Claims 4,5,11(complete and 6-9,12(partially)
Subject relating to the paralogue gene cluster, respectively to the polynucleotide sequences as set forth in SEQ ID NO:12 - SEQ ID NO:16.

The reasons for which the inventions are not so linked as to form a single general inventive concept, as required by Rule 13.1 PCT, are as follows:

Invention 1 discloses the polynucleotide sequences SEQ ID NO:1 (cvm6para), SEQ ID NO:2 (cvm7para) and SEQ ID NO:17 (extended cvm cluster; comprises cvm7 (SEQ ID NO:6)). Said polynucleotides are involved in the production of 5S clavam. Invention 2 discloses polynucleotide sequences (SEQ ID NO: 12 - SEQ ID NO:16; orf paralogue cluster) that are responsible for the production of 5R clavams such as clavulanic acid. Both inventions refer to processes for improving the manufacture of 5R clavams as well as to microorganisms that can be used for such processes.
The general problem underlying the two above mentioned inventions can be seen as the provision of improved methods for the production of 5R clavams. However, this inventive concept is not novel, since already e.g. the subject-matter of WO983396 as well as the subject-matter of WO0130977 relates to methods for improving the production of 5R clavams. Since no other technical feature can be distinguished which might link the subject-matter of said claims, each of the above mentioned groups of claims represents an independent invention.
The application, hence does not meet the requirements of unity of invention as defined

in Rules 13.1 and 13.2 PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V.1 Reference is made to the following documents; D1 as well as D2 have been cited by the Applicant in the description; the numbering will be adhered to in the rest of the procedure:

D1: WO 98/33896 A (UNIV ALBERTA ; SMITHKLINE BEECHAM PLC (GB)) 6 August 1998 (1998-08-06)

D2: MOSHER R H ET AL: "Genes specific for the biosynthesis of clavam metabolites antipodal to clavulanic acid are clustered with the gene for clavaminate synthase 1 in Streptomyces clavuligerus" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC, US, vol. 43, no. 5, May 1999 (1999-05), pages 1215-1224, XP002165205 ISSN: 0066-4804

V.2 Novelty (Article 33(1) and (2) PCT

Claims 1 - 3, 6 - 10 and 12 refer to the polynucleotide sequences cvm6para (SEQ ID NO:1), cvm7para (SEQ ID NO:2) and the extended cvm cluster (SEQ ID NO:17; comprises cvm7 (SEQ ID NO:6)). The subject matter of claims 1 - 3, 6 - 10 and 12 as far as related to SEQ ID NO:1, SEQ ID NO:2 and/or SEQ ID NO:17 is considered as novel since it is not anticipated by the available prior art. Hence, it complies with the requirements of Article 33(1) and (2) PCT.

V.3 Inventive Step (Article 33(1) and (3) PCT)

D1, which is considered to represent the closest prior art, discloses polynucleotide sequences that are specific for the 5S clavam biosynthesis as well as processes for improving 5R production in S. clavuligerus comprising disrupting or otherwise making defective said genes that are essential for the 5S clavam biosynthesis.

The subject-matter referred to in claims 1 - 3, 6 - 10 and 12 differs from D1 in that the open reading frames of the polynucleotide sequences cvm6para and cvm7para are disrupted or deleted such that the production of 5S clavams is reduced and the production of clavulanic acid is at least maintained, respectively improved.

The problem to be solved by the present invention may be regarded as the provision of further polynucleotides that are essential for the production of 5S clavams, respectively

the provision of the further method for improving the production of 5R clavams such as clavulanic acid.
The available prior art (see D2) neither discloses the polynucleotide sequences cvm6para, cvm7para and cvm7 nor suggests that said sequences can be disrupted/deleted in order to improve the 5R clavam production.
Hence, the subject-matter referred to subject matter of claims 1 - 3, 6 - 10 and 12 as far as related to SEQ ID NO:1, SEQ ID NO:2 and/or SEQ ID NO:17 is considered to involve an inventive step (Article 33(3) PCT).

V.4 Industrial Applicability (Article 33(1) and (4) PCT)
The subject matter of claims 1 - 3, 6 - 10 and 12 as far as related to SEQ ID NO:1, SEQ ID NO:2 and/or SEQ ID NO:17 is considered industrially applicable. Hence, it meets requirements of Article 33(1) and (4) PCT.